

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A Method for the specific detection determining the presence or absence of *Mycobacterium tuberculosis* (*M. tuberculosis*) in a biological sample, in which method comprising

(a) a nucleic acid amplification method is carried out using primers which are suitable for amplifying a DNA segment using the nucleic acids from the biological sample as a template and a primer pair capable of amplifying a region of from the sequence shown in SEQ ID NO: 1 which sequence comprises a segment from the region of the *narGHJI* nitrate reductase operon, the DNA segment comprising that encompasses position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon, and

(b) and in the case of which determining the presence or absence of the polymorphism specific for *M. tuberculosis* is detected in position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon, wherein the presence of the polymorphism indicates the presence of *M. tuberculosis* in the biological sample, and the absence of the polymorphism indicates the absence of *M. tuberculosis* in the biological sample.

2. (Currently Amended) The Method according to claim 1, characterised in that wherein the nucleic acid amplification takes place step (a) is carried out by PCR, NASBA, SDA or LCR.

3. (Currently Amended) The Method according to claim 2, wherein characterised in that the PCR is a real time PCR.

4. (Currently Amended) The Mmethod according to any one of claims 1 to 3, characterised in that wherein the detection of the polymorphism specific for M. tuberculosisstep (b) is carried out by the specific hybridisation of one or several probes.

5. (Currently Amended) The Mmethod according to any one of claims 1 to 4, characterised in that wherein the amplified-DNA segment of step (a) has a length of at least 1 and maximum to 500 nucleotides.

6. (Currently Amended) The Mmethod according to any one of claims 1 to 4, characterised in that wherein the amplified-DNA segment of step (a) has a length of at least 1 and maximum to 300 nucleotides.

7. (Currently Amended) The Mmethod according to any one of claims 1 to 4, characterised in that wherein the amplified-DNA segment of step (a) has a length of at least 1 and maximum to 155 nucleotides.

8. (Currently Amended) The Mmethod according to any one of claims 1 to 7, characterised in that wherein one primer of the primer pair of step (a) has at least one of the sequences havingcomprises SEQ ID NO: 2 or SEQ ID NO: 3 or the complementary sequences thereof.

9. (Currently Amended) The Mmethod according to any one of claims 1 to 8, characterised in that wherein the detection of the polymorphism specific for M. tuberculosis takes placestep (b) is carried out by means of at least one pair of labelled hybridisation probes, one probe being labelled at its 3' end and the other probe at its 5' end, and the probes binding specifically to the amplified-DNA segment of step (a) in such a way that a fluorescence resonance energy transfer (FRET) is made possible.

10. (Currently Amended) The Mmethod according to claim 9, wherin one probe of the characterised in that the probe pair has the sequences having comprises SEQ ID NO: 4 and the other probe SEQ ID NO:5; one probe of the probe pair comprises the complementary sequence of SEQ ID NO:4, and the other probe the complementary sequence of SEQ ID NO:5; one probe of the probe pair comprises or the complementary sequences thereof and/or the sequences with SEQ ID NO: 4 and the other probe SEQ ID NO:6; or one probe of the probe pair comprises the complementary sequence of SEQ ID NO:4 and the other probe the complementary sequence of SEQ ID NO:6s thereof.

11. (Currently Amended) The Mmethod according to any one of claims 1 to 10-3, wherin characterised in that the sample is a clinical sample selected from the group of clinical samples consisting of saliva, bronchial lavage, gastric juice, urine, faeces, liquor, bone marrow, blood and biopsies.

12. (Currently Amended) A Pprimer pair -characterised in that it is suitable for the amplification of amplifying a DNA segment from the sequence shown in SEQ ID NO: 1 which that comprises a segment from the area of the narGHJI nitrate reductase operon, wherein the DNA segment comprises position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the narGHJI nitrate reductase operon.

13. (Currently Amended) The Pprimer pair according to claim 12, wherein characterised in that at least one primer of the primer pair of step (a) exhibits the sequence indicated in comprises SEQ ID NO: 2 or the complementary sequence.

14. (Currently Amended) The Pprimer pair according to claim 12, characterised in that at least one primer exhibits the sequence wherein one primer of the primer pair of step (a) comprises indicated in SEQ ID NO: 3 or the complementary sequence.

15. (Currently Amended) The Primer pair according to claim 12, characterised in that the primers exhibit the sequences indicated in wherein one primer of the primer pair of step (a) comprises SEQ ID NO: 2 and the sequences indicated in other primer SEQ ID NO: 3 or the complementary sequences thereof.

16. (Currently Amended) A Primer characterised in that it exhibits the sequence indicated in comprising SEQ ID NO: 2 or the complementary sequence thereof.

17. (Currently Amended) A pPrimer characterised in that it exhibits the sequence indicated in comprising SEQ ID NO: 3 or the complementary sequence thereof.

18. (Cancelled)

19. (Currently Amended) A Hybridisation probe characterised in that it is suitable for the specific detection of detecting the polymorphism specific for *M. tuberculosis* which is located in position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

20. (Currently Amended) A Hybridisation probe pair characterised in that it is suitable for the specific detection detecting of the polymorphism specific for *M. tuberculosis* which is located in position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

21. (Currently Amended) The hHybridisation probe pair according to claim 20, characterised in that at least wherein one probe of the probe pair exhibits the sequence indicated in comprises SEQ ID NO: 4 or the complementary sequence thereof.

22. (Currently Amended) The Hybridisation probe pair according to claim 20, characterised in that at least wherein one probe of the probe pair exhibits the sequence indicated in SEQ ID NO: 5 or the complementary sequence thereof.

23. (Currently Amended) The Hybridisation probe pair according to claim 20, characterised in that at least wherein one probe of the probe pair comprises exhibits the sequence indicated in SEQ ID NO: 6 or the complementary sequence thereof.

24. (Currently Amended) The Hybridisation probe pair according to claim 20, characterised in that the probes exhibit the sequences indicated wherein one probe in the probe pair comprises SEQ ID NO: 4 and the other probe SEQ ID NO: 5, or one probe in the probe pair comprises the complementary sequence of SEQ ID NO:4 and the other probe the complementary sequence of SEQ ID NO:5 thereof.

25. (Currently Amended) The Hybridisation probe pair according to claim 20, characterised in that the probes exhibit the sequences indicated in wherein one probe in the probe pair comprises SEQ ID NO: 4 and the other probe SEQ ID NO: 6, or one probe in the probe pair comprises the complementary sequences thereof of SEQ ID NO:4 and the other probe the complementary sequence of SEQ ID NO:6.

26. (Currently Amended) A Hybridisation probe characterised in that it exhibits the sequence indicated in comprising SEQ ID NO: 4 or the complementary sequence thereof.

27. (Currently Amended) A Hybridisation probe characterised in that it exhibits the sequence indicated in comprising SEQ ID NO: 5 or the complementary sequence thereof.

28. (Currently Amended) ~~A~~ ~~H~~ybridisation probe characterised in that it exhibits the sequence indicated ~~incomprising~~ SEQ ID NO: 6 or the complementary sequence thereof.

29. (Canceled)

30. (Canceled)

31. (Currently Amended) ~~A~~ ~~K~~it according to claim 30 for detecting *M. tuberculosis*, which ~~comprises~~ comprising:

-at least one primer pair suitable which is suitable for the amplification of amplifying a DNA segment from the sequence shown in SEQ ID NO: 1 which comprises a segment from the area of the *narGHJI* nitrate reductase operon, wherein the DNA segment comprising ~~comprises~~ position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon, and/or

~~comprises~~ at least one hybridisation probe or a hybridisation probe pair which is suitable for the specific detection of detecting the polymorphism specific for *M. tuberculosis* which is located in position -215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

32. (Currently Amended) The ~~K~~it according to claim 31, characterised in that wherein the primer pair is the primer pair according to claim 15.

33. (Currently Amended) The ~~K~~it according to claim 31, wherein characterised in that the hybridisation probe pair is the probe pair according to claim 24 and/or the probe pair according to or claim 25.

34. (Currently Amended) ~~The kit according to claim 33, further comprising~~
characterised in that the primer pair is the primer pair according to claim 15.

35. (Currently Amended) ~~The kit according to claims 30 to 34 or claim 32, characterised in that it comprises further comprising reagents and/or auxiliary agents necessary or useful for carrying out a nucleic acid amplification and/or detection reaction.~~

36. (Currently Amended) ~~A method for the specific detection/determining the presence or absence of *mycobacterium tuberculosis* (*M. tuberculosis*) in clinical material, comprising the steps~~

a) ~~extraction of~~ extracting microbial DNA from clinical material,
b) ~~amplification of~~ amplifying from the extracted DNA at least one DNA fragment of the promoter region of the *narGHJI* nitrate reductase operon of mycobacteria containing at least one DNA polymorphism specific for *M. tuberculosis* from the extracted DNA, and

c) ~~detection of~~ determining the presence or absence of the specific hybridisation of the ~~amplified~~ DNA fragment of step (b) by way of melting curve analysis with at least one hybridisation probe which ~~that~~ comprises the nucleotide sequence selected from the group consisting of SEQ ID NO: 5, the complementary sequence to SEQ ID NO: 5, SEQ ID NO: 6, and the complementary sequence to SEQ ID NO: 6,

~~the specific detection of *M. tuberculosis* vis à vis *M. bovis*, *M. bovis* BCG, *M. africanum* and *M. microti* taking place by way of melting curve analysis, wherein the presence of the specific hybridization indicates the presence of *M. tuberculosis* in the clinical material, and the absence of the specific hybridization indicates the absence of *M. tuberculosis* in the clinical material.~~

37. (Currently Amended) ~~The method according to claim 36, wherein the specific hybridization is based on the DNA polymorphism being located in position -215 of the~~

promoter region in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

38. (Currently Amended) The Mmethod according to claim 37, the amplification taking place in wherein step b) is carried out by means of a primer pair having one primer that comprising the nucleotide sequences SEQ ID NO: 2 and the other primer /SEQ ID NO: 3.

39. (Currently Amended) The Mmethod according to any one of claims 36 to 38, the specific hybridisation taking place in wherein step c) is carried out with at least one pair of labelled hybridisation probes, and wherein one probe of the pair comprising the nucleotide sequences SEQ ID NO: 4 and the other /SEQ ID NO: 5, one probe of the pair comprises or the pair of the complementary sequences thereof of SEQ ID NO:4 and the other the complementary sequence of SEQ ID NO:5, one probe of the pair comprises SEQ ID NO:4 and the other SEQ ID NO:6, and/or one probe of the pair comprises the nucleotide sequences the complementary sequence of SEQ ID NO: 4 and the other the complementary sequence of /SEQ ID NO: 6 or the pair of complementary sequences thereof.

40. (Currently Amended) The Mmethod according to any one of the preceding claims 36 to 38, the multiplication of the DNA fragments wherein step (b) is carried out by polymerase chain reaction (PCR) being carried out preferably as real time PCR, preferably be means of the LightCyclerTM system.

41. (Currently Amended) The Mmethod according to any one of one of the preceding claims 36 to 38, the steps of specific hybridisation taking place wherein step (c) is carried out during or after the amplification of the DNA fragments step (b).

42. (Currently Amended) The Method according to any one of the preceding claims 36 to 38, wherein the specific hybridisation and its detection taking place in step (c) is carried out via real time PCR, preferably in the LightCycler™ system.

43. (Currently Amended) The Method according to any one of the preceding claims 36 to 38, the detection of the specific hybridisation being wherein step (c) is carried out by fluorescence detection, and the labelled hybridisation probe pairs being are formed as fluorescence resonance energy transfer (FRET) pair.

44. (Currently Amended) The Method according to any one of the preceding claims 36 to 38, wherein the clinical material being is selected from the group of clinical samples consisting of saliva, bronchial lavage, gastric juice, urine, faeces, liquor, bone marrow, blood and biopsies.

45. (Currently Amended) An Oligonucleotide primer pair for the amplification of amplifying a DNA fragment of the *M. tuberculosis*-specific promoter region of the *narGHJI* nitrate reductase gene, wherein one primer in the primer pair comprising comprises the nucleotide sequences SEQ ID NO: 2, and the other primer /SEQ ID NO: 3.

46. (Currently Amended) An Oligonucleotide which hybridises specifically with an *M. tuberculosis*-specific promoter region of the *narGHJI* nitrate reductase operon, comprising the nucleotide sequence SEQ ID NO: 5 or the complementary sequence thereof.

47. (Currently Amended) An Oligonucleotide which hybridises specifically with an *M. tuberculosis*-specific promoter region of the *narGHJI* nitrate reductase operon, comprising the nucleotide sequence SEQ ID NO: 6 or the complementary sequence thereof.

48. (Currently Amended) An Oligonucleotide pair, wherein one oligonucleotide in the pair comprising the nucleotide sequences SEQ ID NO: 4 and the other

oligonucleotide /SEQ ID NO: 5, or the pair of one oligonucleotide in the pair comprises the complementary sequences thereof of SEQ ID NO:4 and the other oligonucleotide the complementary sequence of SEQ ID NO:5.

49. (Currently Amended) An Oligonucleotide pair, wherein one oligonucleotide in the pair comprising the nucleotide sequences comprises SEQ ID NO: 4 and the other /SEQ ID NO: 6, or one oligonucleotide in the pair of comprises the complementary sequences thereof of SEQ ID NO:4 and the other the complementary sequence of SEQ ID NO:6.

50. (Currently Amended) A kit for the specific detection of detecting *Mycobacterium tuberculosis* comprising

- a) at least one primer pair, wherein one primer in the primer pair comprising comprises the nucleotide sequences SEQ ID NO: 2, and the other primer /SEQ ID NO: 3 and
- b) at least one hybridisation probe pair, wherein one probe in the probe pair comprising comprises the nucleotide sequences SEQ ID NO: 4 and the other probe /SEQ ID NO: 5, one probe in the probe pair comprises the complementary sequence of SEQ ID NO:4 and/or the pair of other probe the complementary sequence of SEQ ID NO:5, one probe in the probe pair comprises s thereof and/or at least one hybridisation probe pair comprising the nucleotide sequences SEQ ID NO: 4 and the other probe /SEQ ID NO: 6, or the pair of one probe in the probe pair comprises the complementary sequences thereof of SEQ ID NO:4 and the other probe the complementary sequence of SEQ ID NO:6.

51. (Currently Amended) Use of a *M. tuberculosis* specific DNA polymorphism in the promoter region of the *narGHII* nitrate reductase operon of mycobacteria containing the nucleic acid sequence represented in The method of claim 36, wherein the at least one hybridization probe in step (c) comprises SEQ ID NO: 6 or the complementary sequence thereof, for the specific detection of an infection with *M. tuberculosis*.